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Chromatographic and Spectrophotometric Characterization of Adducts Formed During the Reaction of *trans,trans*-Muconaldehyde with ^{14}C-Deoxyguanosine 5′-Phosphate

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Mice liver microsomes oxidatively open the benzene ring to form *trans,trans*-muconaldehyde, a hematotoxic unsaturated aldehyde. In the present studies, 4.5 μmole *trans,trans*-muconaldehyde was reacted with ^{14}C-2′deoxyguanosine 5′-phosphate in phosphate buffer. Products were separated by high performance liquid chromatography (HPLC). Absorbance was monitored using a diode array detector, and aliquots of the HPLC eluant were collected for UV spectrophotometric analysis and scintillation counting. Under these conditions, deoxyguanosine 5′-phosphate eluted at 12.5 min and muconaldehyde at 22.0 min. The HPLC and radioactivity profiles of the muconaldehyde/deoxyguanosine reaction mixture indicated the presence of multiple adducts. Three adducts were detected eluting at 36, 39, and 42 min, which represented approximately 2.5, 2.5, and 1% of the radioactivity, respectively. These adducts had similar UV spectra with absorption maxima between 334 and 347 nm. Another product of the reaction mixture, eluting at 19.0 min and accounting for 10% of the radioactivity, was also observed. This compound had absorption maxima at 348 and 372 nm. These results suggest that *trans,trans*-muconaldehyde can react with deoxyguanosine monophosphate *in vitro* under physiological conditions to form stable adducts. Studies are being conducted to determine the structure of these adducts and whether these adducts are formed by the reaction of DNA with muconaldehyde or metabolically activated benzene.

Introduction

trans,trans-Muconaldehyde (TTM), an α,β-unsaturated diene dialdehyde, has been suggested by Goldstein et al. (*1*) to be a toxic intermediate in the metabolism of benzene to *trans,trans*-muconic acid. Since the early studies of Parke and Williams it has been known that benzene is metabolized to this ring-opened dicarboxcylic acid (*2*). Only recently, however, has TTM been identified as a product of benzene metabolism in mouse liver microsomes (*3*). Enzymatic oxidation of muconaldehyde to *trans,trans*-muconic acid by aldehyde dehydrogenase has been shown to occur in both rat and mouse liver cytosol (*4*). It has also been demonstrated that TTM is a potent hematotoxin. Administration of 3 mg/kg daily for 16 days to CD-1 mice resulted in statistically significant decreases in erthrocyte count, hematocrit, and bone marrow cellularity (*5*).

The present work describes the formation of adducts by the reaction of TTM with deoxyguanosine 5′-phosphate. This study is the first step in determining whether muconaldehyde-derived DNA adducts are important in the carcinogenicity of benzene.

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Materials

8-^{14}C-2′-Deoxyguanosine 5′-phosphate was obtained from Schwarz/Mann and diluted with unlabeled deoxyguanosine 5′-phosphate (Sigma Chemical Co.) to a specific activity of 0.82 mCi/mmole. TTM was synthesized according to the method of Kossmehl and Bohn (*6*) as previously described (*3*).

Methods

Reaction conditions. One milligram (4.5 μmole) of TTM was added directly to 6 mg (9 μmole) 8-^{14}C-2′-deoxyguanosine 5′-phosphate in 2 mL of 10 mM phosphate buffer, pH 7.4, and the mixture was incubated at 37° for 16 hr.

HPLC Analysis. Products from the reaction mixture were separated by high performance liquid chromatography (HPLC) using a Phenomenex C-18 μBondapak column and a gradient of 10 mM phosphate or 200 mM ammonium acetate, pH 5.0, from 0 to 60% methanol over a period of 60 min. Absorbance was monitored on-line from 220 to 370 nm with an LKB diode array detector. During HPLC analysis, 1 mL aliquots were collected to which were added 10 mL of Hydrofluor scintillation cocktail (National Diagnostics), and their associated radioactivity was determined by using an LKB Rackbeta liquid scintillation counter. In addition, the UV spectra of radioactive compounds eluting from the HPLC were analyzed using a Perkin-Elmer Lambda 3B spectrophotometer.

Results and Discussion

As illustrated in Figure 1, the chromatogram and radioactivity profile obtained after HPLC analysis of the reaction of TTM with deoxyguanosine 5′-phosphate contain several major adduct peaks that are not observed in the deoxyguanosine 5′-phosphate control (Fig. 2). An exami-

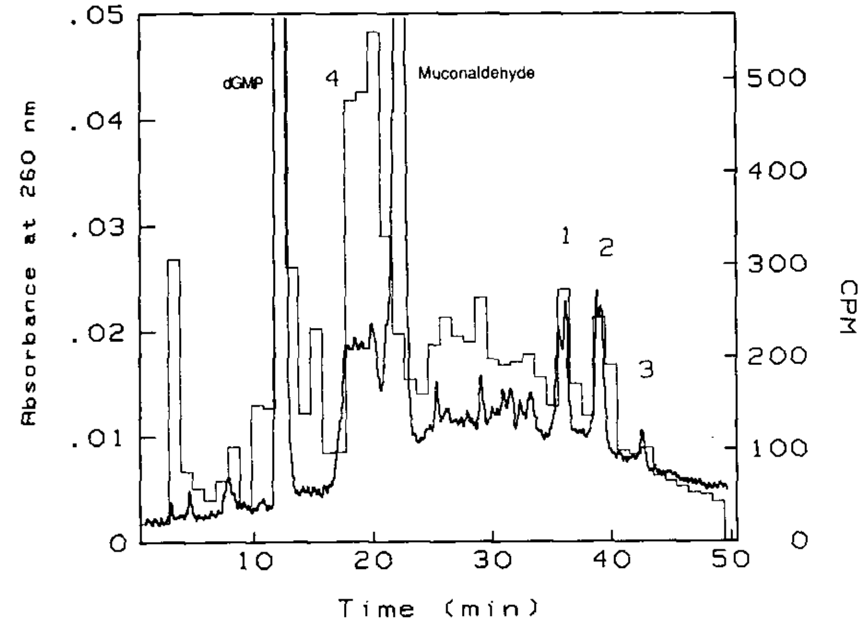

FIGURE 1. Chromatogram and radioactivity profile of TTM with ^{14}C-deoxyguanosine 5′-phosphate. Injection volume was 20 μL; mobile phase was a linear gradient of 10 mM phosphate, pH 5.0, and methanol. For other conditions see text.

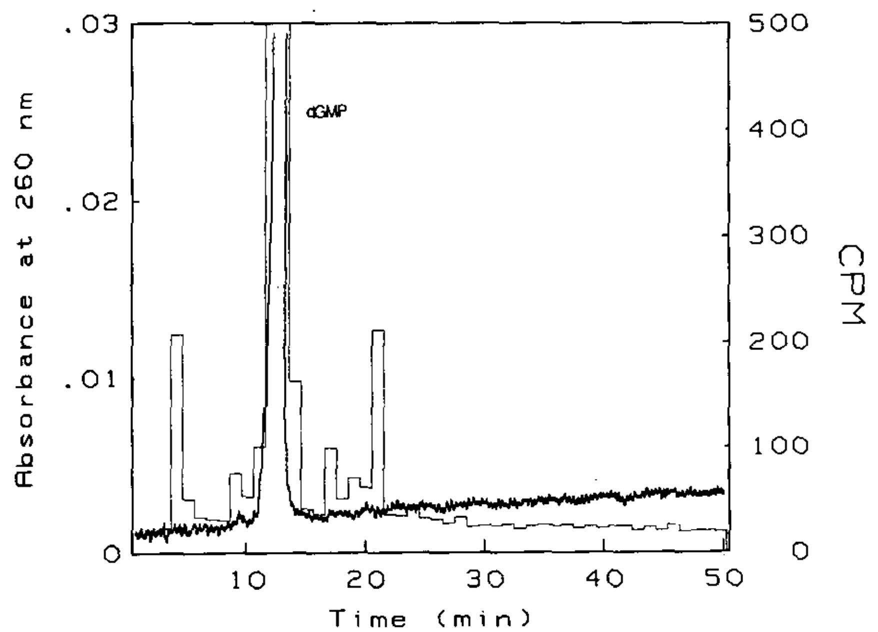

FIGURE 2. Chromatogram and radioactivity profile of ^{14}C-deoxyguanosine 5′-phosphate. Injection volume was 10 μL; mobile phase was a linear gradient of 10 mM sodium phosphate, pH 5.0, and methanol. For other conditions see text.

nation of the UV spectra of these adducts reveals the following: adducts 1, 2, and 3, eluting at 26, 39, and 42 min, have absorption maxima at 334, 342, and 347 nm, respectively (Fig. 3*A*–*C*). Adduct 1 has an additional maximum at 272 nm, while adducts 2 and 3 have a shoulder at this wavelength and another shoulder at 254 nm. In the chromatogram of the muconaldehyde/deoxyguanosine mixture at 260 nm (Fig. 1), adducts 1 and 2 appear to be split peaks. Expansion of the chromatogram in this region and on-line spectral analysis reveals that the spectra of the material under each half of the split peaks are identical. This finding suggests that these adducts may be a mixture of diastereomers.

In addition to the these adducts, there are several other radioactive peaks in this chromatogram that are not present in the control. The most prominent of these peaks is the irregularly shaped peak eluting at 19 min (Fig. 1, no. 4). As shown in Figure 3*D*, this peak has an absorption maximum at 248 nm and a shoulder at 272 nm. In subsequent analysis of the reaction mixture, this peak assumed a more symmetrical shape.

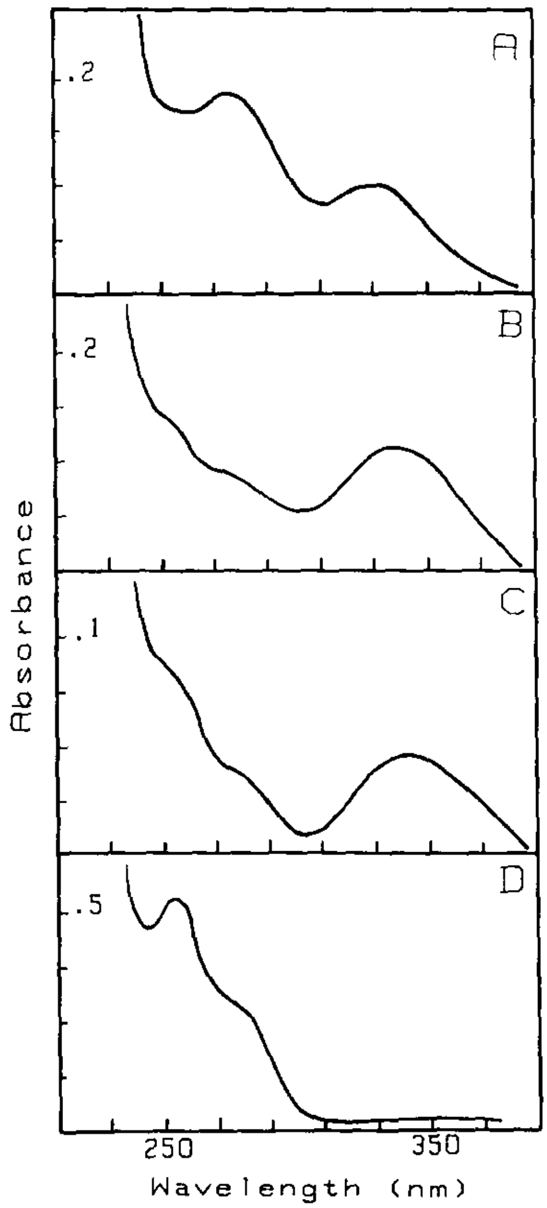

FIGURE 3. UV spectra of peaks 1–4 collected from the HPLC in 1- to 2-mL aliquots (injection volume 250 μL). Samples were in 200 mM ammonium acetate: methanol. (*A*) Adduct 1, retention time, 36 min; (*B*) adduct 2, retention time, 39 min; (*C*) adduct 3, retention time, 42 min; (*D*) adduct 4, retention time, 19 min.

The percent yields of the major adducts, based on the radioactivity associated with ^{14}C-deoxyguanosine, are approximately 2.5% for each the possible diastereomers of adducts 1 and 2, 1% for adduct 3, and 14% for adduct 4. Two other peaks with retention times of 26 and 29 min are associated with a small amount of radioactivity, but not enough material was present for a UV spectral analysis.

These studies demonstrate that TTM reacts under physiological conditions to form adducts of variable stability. Diastereomeric cyclic 1,N^2-deoxyguanosine adducts have been reported by other investigators (*7,8*) to be the major products formed during the reaction of other α,β-unsaturated aldehydes with deoxyguanosine. The cyclic 1,N^2-propanodeoxyguanosine adduct formed during the reaction of acrolein and crotonaldehyde with deoxyguanosine (*7*) has chromatographic and spectral characteristics similar to that of the major muconaldehyde-deoxyguanosine adduct (no. 4). The finding that the other adducts (nos. 1–3) have significant absorbance at longer wavelengths may reflect the extended conjugation system of muconaldehyde, which absorbs maximally at 272 nm. Studies are underway to determine the structure of the adducts formed during the reaction of muconaldehyde with deoxyguanosine, as well as to determine whether similar adducts are formed by the reaction of DNA with muconaldehyde or metabolically activated benzene.

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